Inflammation:
Where Immune Cells and Blood Vessels Collide

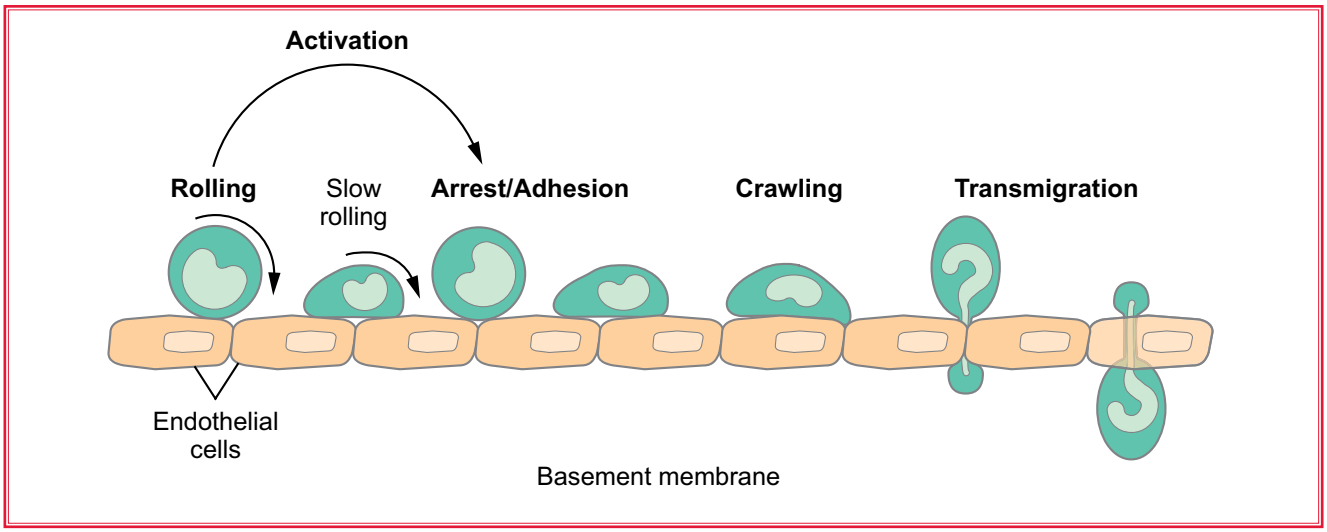
In an average person, there are approximately 20,000,000,000 neutrophils patrolling the bloodstream on the lookout for trouble. These foot soldiers of the immune system represent a first line of defense against foreign assault from a variety of pathogens. As they circulate through the body, neutrophils might find themselves slowing down in thickening blood and brushing up against blood vessel walls where the tissue has been inflamed by infection or disease. This first casual interaction with the endothelial cells that line blood vessels begins a complex cascade of intercellular molecular interactions that ultimately propels the neutrophils out of the vasculature and into the damaged tissue where they can wreak havoc on the enemy before committing hara-kiri. Neutrophils are just one of several types of white blood cells—or leukocytes—that must interact with vascular endothelial cells to execute their immune functions. Triantafyllos Chavakis, M.D., Ph.D., Head of the Inflammation Biology Section in CCR's Experimental Immunology Branch, is studying the molecular basis of leukocyte-endothelial interactions with the goal of finding ways to suppress the damaging inflammatory response that characterizes inflammatory and autoimmune diseases.

Tissue becomes inflamed—whether because of a bug bite or arthritis—when chemical signals elicit the blood vessel changes necessary to recruit leukocytes to the site of injury. Neutrophils, for example, have specific receptors that detect and respond to certain bacterial proteins; other leukocytes respond to chemical signals known as chemokines that are released by cells in the area as a kind of general alarm. Although inflammation does not literally set tissue on fire, like its namesake, it must be deployed carefully lest it cause more harm than good. Autoimmune diseases such as multiple sclerosis and rheumatoid arthritis are caused by a misguided attack from the immune system on host tissue, which includes inflammation as a key destructive force. Neurodegenerative diseases like Alzheimer's also appear to have a strong inflammatory component. In such cases, although inflammation itself may not be the root cause of the disorder, stopping it can be a strong defense. Indeed, leukocyte inhibitors are already used to treat psoriasis and multiple sclerosis. "Whether you study inflammatory disease or autoimmunity," noted Chavakis, "an essential component is the leukocyte—if you block this, you could target disease."

The Leukocyte-Endothelial Interaction Cascade

Endothelial cells maintain a physical barrier that allows for the efficient circulation of blood as well as metabolic exchange with surrounding tissue. Leukocytes are free to travel these passageways, but leaving them requires an active multistep molecular signaling cascade that engages both the leukocyte and the endothelial cells.

The process by which a leukocyte leaves the bloodstream to enter a tissue is generally thought of as occurring in three



(Image: J. Kelly)

The leukocyte-endothelial interaction cascade. Leukocytes leave the blood vessels through a multistep process beginning with rolling and ending in transmigration through the endothelial cells.

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phases: rolling or tethering, activation, and then adhesion and transmigration, which have both mechanical and molecular signaling aspects. In the first phase, a leukocyte will literally bump up against the endothelial cells forming the blood vessel. This interaction results in a weak binding between molecules known as selectins on the surface of the leukocyte and their counterparts on the endothelial cells. Once loosely tethered through selectin-binding, leukocytes are exposed to chemokines at the endothelium that are produced during inflammation. Chemokines then activate the leukocytes to bind more tightly to the endothelial surface through a different set of receptors—integrins. Integrin-binding enables the leukocytes to crawl along the blood vessel seeking a point of exit. More often than not, this exit occurs at a junction between endothelial cells where a different set of molecular interactions guides the leukocyte through the normally sealed barrier.

Cascade Inhibitors Identified

As a doctoral and postdoctoral student and later as a practicing clinician, Chavakis conducted research in the laboratory of Klaus Preissner, Ph.D., in Germany. His interest in the biology of leukocyte-endothelial interactions was stimulated, in part, by the patients he saw, including many with diabetic complications such as wounds that refused to heal and that became chronically infected by bacteria. However, his research was inspired not as a direct challenge to infection but from a desire to copy bacterial strategies for subverting inflammation. "We weren't really studying how innate immunity copes with bacteria," remembered Chavakis, "but how bacteria can avoid the innate immune response."

The bacteria *Staphylococcus aureus* have adapted themselves to successful human infection through a range of strategies including the production of factors that interact with host proteins to assist in bacterial colonization and propagation. In 2002, Chavakis and his colleagues identified Eap (extracellular adherence factor)—a protein secreted by bacteria to block the innate immune response by inhibiting recruitment of neutrophils. They found that Eap interacted with ICAM-1 to prevent the adhesion of leukocytes required for their translocation to the site of infection. They went on to demonstrate that Eap could dampen the autoimmune response in a mouse model of multiple sclerosis. In the meantime, *Staphylococcus* has been found to produce inhibitors that target each step in the leukocyte-endothelial interaction cascade.

Upon moving to NCI, Chavakis shifted his focus away from bacterial effectors to study intrinsic mechanisms of innate immunity. However, he was surprised to discover that leukocyte inhibitors were not so easily dismissed. In a paper published in *Science* in 2008, Chavakis and his colleagues demonstrated that a previously known glycoprotein—Del-1 (Developmental endothelial locus-1), which had been implicated in blood vessel remodeling—had an important novel role in leukocyte-endothelial adhesion.

"If you look at the leukocyte adhesion cascade," explained Chavakis, "you will find maybe 20–30 receptors generally working to promote adhesion. Very few adhesion proteins do the opposite." Although Del-1 had all the hallmarks of a protein that bound to the adhesion machinery of leukocytes, it appeared to block adhesion instead of promoting it. They found that mice lacking the *Del-1* gene demonstrated increased leukocyte adhesion and accumulation of neutrophils when challenged in a model of lung inflammation. Furthermore, the researchers were able to show that Del-1 was produced at high levels in parts of

the body that tightly restrict access by the immune system, like the brain and the eyes.

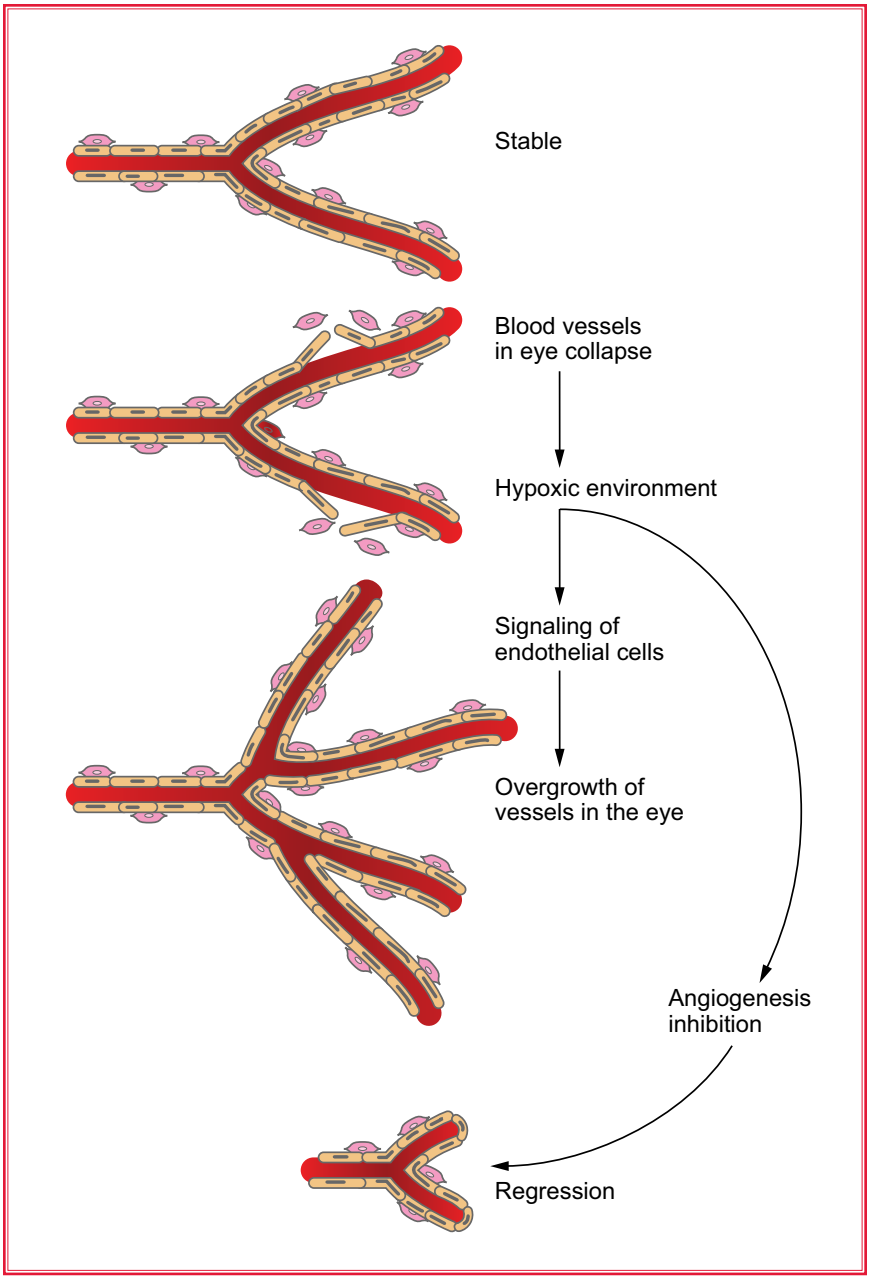
Currently, they are challenging the *Del-1* knockout mice in a model of multiple sclerosis. "But it would be even more interesting if we could generate bioavailable forms of the protein and see if it could really inhibit inflammatory disease," noted Chavakis. The team is currently working with NCI's Protein Purification Laboratory to render the Del-1 that they are producing in cell cultures fit to test in animal models.

A Small Step to Angiogenesis

From studying the vascular changes that occur during inflammation, it is not a large experimental leap to study the formation of new blood vessels in developed tissue since both involve initial changes in vascular permeability and many of the same molecular factors. One of the key initiators of vascular growth is a lack of oxygen.

Chavakis's lab works with a particular model of retinopathy in mice that has strong parallels with a disorder that occurs in babies born prematurely. Because their lungs are not sufficiently developed, these babies often require high oxygen environments, which unfortunately damage the retinal vasculature. In mice, the retinal vasculature develops normally in the first 15 days after birth, but a high oxygen environment in the second week destroys the retinal vessels. Once you return the mice to a normal oxygen environment, the resulting hypoxia causes the disorganized pathological vessel growth that is characteristic of many other forms of retinopathies as well as cancers. This model is both physiologically faithful to human disease and nicely accessible to study; in collaboration with researchers at the National Eye Institute, Chavakis hopes to be able to inject compounds directly into the eye to observe their effects on neovascularization.

Chavakis estimates that his efforts are currently split two-thirds/one-third between leukocyte-endothelial interactions and angiogenesis, but his interest in both is stimulated by the clear overlap between the two areas of study. Recently, a molecule that Chavakis helped to identify while he was still in Germany for its role in leukocyte transmigration has reappeared in the laboratory under the more general guise of altering vascular permeability.



(Image: J. Kelly)

Model of hypoxia-induced retinopathy. The collapse of blood vessels in the eye results in a lack of oxygen delivery to the tissue. This hypoxic environment generates signals that cause abnormal blood vessel proliferation, which can be suppressed by angiogenesis inhibitors.

Junctional adhesion molecule-C or JAM-C, as it is now known, first came to Chavakis's attention through a colleague, Sentot Santoso, Ph.D., who was immunizing mice with human platelets in order to study the molecules responsible for an autoimmune disorder known as immune thrombocytopenia. "He had tons of nice antibodies and some of them reacted with new unknown targets," recalled Chavakis. The two researchers began talking and eventually working together when it became apparent to Dr. Santoso that one of the targets had a sequence very similar to the only known junctional adhesion molecule at the time. "So we immediately picked it up to see if it was in endothelial cells and, in fact, the first thing that we published was that it binds to integrins on leukocytes." They and others have gone

on to demonstrate that JAM-C localizes to junctions between endothelial cells and regulates the ability of leukocytes to pass between them.

In a paper published in *The Journal of Experimental Medicine* in 2006, Chavakis's group went on to demonstrate that JAM-C has a somewhat counterintuitive role in inhibiting adhesion between endothelial cells. "We found that JAM-C regulated junctions in the opposite manner: by removing the protein, the junctions became better." Disrupting JAM-C caused a decrease in permeability and a dampening of the normal increase in permeability caused by histamine or vascular endothelial growth factor (VEGF). It also decreased the extent of aberrant new blood vessels in their mouse model of retinopathy. Chavakis and his colleagues are currently working on a conditional knockout of the gene in mice to further study its functions *in vivo*.

(*Photo: R. Baer*)

From left to right: Triantafyllos Chavakis, M.D., Ph.D.; Harald Langer, Ph.D.; Eun Young Choi, Ph.D.; and Valeria Orlova, Ph.D. Missing from the Chavakis team photo: Sunil Kaul, Ph.D.

Homeostasis: What Is Normal?

Chavakis is intrigued by the fact that, depending on the tissue examined, the blood vessel endothelium can have vastly different functions. In the lung, it must regulate oxygen transfer and resist the temptation to mount an inflammatory response to every foreign agent that is inhaled. In the brain, it must protect the neural tissue from a variety of molecules that are free to pass through virtually any other tissue in the body. The blood-brain barrier also resists invasion by cells of the immune system, whereas, in the liver, the blood vessels are open and fenestrated to allow a much greater exchange of molecules. "Obviously, the endothelium in each case is adapted to the function of the tissue," commented Chavakis. "But why and how?" These tissue differences are only starting to be considered experimentally, yet it is likely that the leukocyte-endothelial interaction cascade itself is different depending on the tissue type.

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"There is a perception that you easily adopt if you read big reviews in this field that leukocytes themselves only promote vascular growth, but if you start doing experiments, you sometimes end up with different results," noted Chavakis. "And then, there is a smaller piece of literature, which is easy to ignore, showing that certain leukocytes probably do the opposite." Chavakis is more and more convinced that these seemingly contrary findings will prove important to understanding vascular integrity.

More difficult to study than the processes stimulated by inflammation, Chavakis wants to find a way to study the normal homeostatic mechanisms that maintain blood vessels. In the absence of infection or disease, blood vessels are generally quiet. Unlike many organ systems that have a high turnover of cells in their tissue, healthy blood vessels maintain their integrity without much fanfare, resting shoulder to shoulder to provide a safe passageway for the blood. However, this in itself is a mystery—how do blood vessels maintain their integrity in the face of the physical stresses they encounter? Every day, all day, blood cells stream along the vessel lining, yet they do not wear it down. Chavakis is convinced that some of the molecules he studies could be actively involved in maintaining this perceived quiescence.

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The challenge will be to study it. "In contrast to conditions like tumor angiogenesis or retinopathy, how do you create situations to manipulate vascular maintenance in which you don't address proliferation? We are still trying to find out how to do it." In the meantime, Chavakis will continue to mine the rich intersection between immune and vascular function. "Immunologists don't consider vascular biology, and vascular biologists don't study immune systems. A few of us are happy to be somewhere in the middle."

To read more about Dr. Chavakis's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?profileid=10637.